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(54) Title: ONE-STEP FLUORESCENT IMMUNOSENSOR TEST

(57) Abstract

An immunoassay for detecting the presence of an analyte in a sample solution is disclosed. The immunoassay includes a nitrocellulose solid support matrix with a sample zone, a label zone and a capture zone. A solution of fluorescent latex beads having a lanthanide chelate in association with antibodies against the target analyte is disposed onto the label zone.

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ONE-STEP FLUORESCENT IMMUNOSENSOR TEST

Field of the Invention

This invention relates to systems and methods for detecting analytes in biological fluids. More specifically, this invention relates to one-step immunoassays that use fluorescent latex microspheres containing a europium chelate as the labeling agent.

Background of the Invention

Analyte-specific binding assays are important tools for detecting and measuring environmental and biologically relevant compounds, including hormones, metabolites, toxins and pathogen-derived antigens. A convenient version of the binding assay is an immunoassay which can be conducted in a "lateral flow" format.

Devices useful for performing lateral flow assays typically include solid support matrices, such as nitrocellulose, having several "zones" that are defined along a length of the matrix. The matrix defines a flow path and provides fluid connection between the various zones, including a sample receiving zone, a labeling zone for specifically labeling the analyte, and a capture (detection) zone located downstream from the sample receiving zone and the labeling zone. An absorbent zone (sink) typically is located downstream of the capture zone, and provides a means for removing excess sample and unbound label from the matrix.

A principal advantage of the lateral flow immunoassay is the ease with which the testing procedure is carried out. In this procedure a sample solution containing the analyte to be detected is placed onto a sample receiving zone in the matrix. Capillary action then draws the liquid sample and analyte downstream into a labeling zone. The labeling zone normally contains a labeled immunoglobulin that specifically attaches to the target analyte in the sample solution.

After flowing through the labeling zone, the sample solution continues to flow into the capture zone where it contacts an immobilized compound capable of specifically binding the labeled target analyte. As a specific example, analyte-specific immunoglobulins can be immobilized to the matrix in the capture zone. Labeled target analytes become trapped by the immobilized immunoglobulins within the capture zone, thus becoming visually or otherwise detectable. Finally, the procedure completes when the excess sample is taken up by the material of the absorbent zone.

There are two principal types of one-step immunoassays. The first, a "wet" one-step immunoassay includes a solid support matrix having a sample zone, capture zone and absorbent zone. In this system, the sample and a targeted label are mixed prior to contacting the solid support matrix. The targeted label, such as a colorimetrically labeled antibody, specifically binds with any analyte in the sample solution prior to being placed on the solid support matrix. After the sample solution is placed on the sample zone of the solid support matrix, the sample solution moves by capillary action across the capture zone wherein the analyte becomes fixed to bound antibodies in the capture zone. Because the analyte is colorimetrically labeled, the capture zone changes colors if any analyte is present in the solution.

The second type of one-step bioaffinity assay is a "dry" assay, wherein the solid support matrix includes a sample zone, labeling zone, capture zone and absorbent zone. Thus, this assay differs from a wet bioaffinity assay by including label directly on the solid support matrix. A sample solution containing the analyte of interest is first placed on the sample zone. Through capillary action, the sample solution traverses the solid support matrix. As the analyte in the sample passes the label zone, any analyte becomes labeled. The sample solution then continues to move across the solid support matrix to the capture zone. As discussed above, the capture zone includes bound antibodies that prevent the analyte from continuing to the move through the matrix. A positive result is seen when label accumulates in the capture zone and results in a color change.

Although many types of label have been used within the label zone of immunoassays to allow detection of the target analyte, many difficulties still exist in quickly detecting small quantities of particular analytes within a sample. For example, others have resorted to using radioactive isotopes such as ^{32}P or ^{35}S to detect small quantities of a particular analyte. Unfortunately, the detection process for radioactive isotopes can take a very long time. In addition, radioactive labels are unappealing to many people because of their potential for causing health problems.

U.S. Patent No.: 5,451,504 to Fitzpatrick et al. discusses the use of colorimetric latex beads to label samples in an immunoassay for detecting benzoylecgonine in a urine sample. However, colorimetric beads are known to be relatively insensitive. Thus, minute quantities of a particular analyte cannot normally be detected with colorimetric labels. For example, colorimetric beads can normally only detect analyte in quantities of greater than 2-4 nanograms/ml.

Fluorescent labels have been used within some types of multistep immunoassay systems, but their sensitivity has been limited due to the background fluorescence of the naturally-fluorescing matrix constituents. To combat this problem, some investigators have developed time resolution techniques that isolate the specific signal of interest from the background signals. Time resolution techniques have been attempted in multi-step immunoassays using some aromatic diketone chelates, such as europiumbenzoylacetonate and europiumbenzoyl-trifluoroacetonatebecause of their long-lived fluorescence. In addition, the aromatic diketone chelates have been incorporated into polymeric bead lattices to reduce the quenching of fluorescent signals by aqueous solutions (U.S. Patent No.: 4,283,382).

Unfortunately, these techniques still utilize time resolution techniques for determining whether the fluorescent signal was generated from a bound analyte, or from background fluorescence. Because time resolution techniques take a long time to complete, more convenient and efficient one-step immunoassays are still needed in the art.

Thus, there exists an unmet need in the art for an immunoassay system that is simple, rapid and sensitive enough to quickly detect minute quantities of an analyte. The present invention provides a solution for this problem.

Summary of the Invention

One embodiment of the invention is a one-step immunoassay device, including: a solid support matrix having a sample zone, a label zone and a capture zone; and at least one fluorescent latex microsphere containing a lanthanide chelate disposed on the label zone.

Another embodiment of the invention is a lateral flow immunoassay device having a solid support matrix. The immunoassay device includes: a sample zone; a label zone in fluid communication with the sample zone, the label

zone further comprising at least one fluorescent latex microsphere containing a lanthanide chelate in communication with an antibody having specificity for an analyte; and a capture zone in fluid communication with the label zone, the capture zone containing a capture antibody having affinity for the analyte.

Yet another embodiment of the invention is a method for detecting the presence of an analyte in a sample. The method includes the steps of: a) contacting a sample zone on a solid support matrix with a sample to be tested for the presence of an analyte, wherein the sample moves along the solid support such that the sample contacts a label zone comprising antibodies with specificity for the analyte in association with fluorescent latex microspheres having a lanthanide chelate, and wherein the analyte, the antibodies and the fluorescent latex microspheres form a fluorescent complex; and b) detecting the presence of the fluorescent complex, wherein the detection of the fluorescent complex is indicative of the analyte being present in the sample.

Still another embodiment of the invention is a method for detecting the presence of an analyte in a sample solution. This method includes the steps of: placing a sample solution onto a sample zone of a solid support matrix, the solid support matrix comprising the sample zone, a label zone and a capture zone, wherein and at least one fluorescent latex microsphere containing a lanthanide chelate is disposed on the label zone; waiting a pre-determined time for the sample to move across the solid support to the capture zone; and detecting the presence of an analyte in the sample solution by measuring the amount of fluorescence on the capture zone.

Still another embodiment of the invention is a method for detecting the presence of an analyte in a sample solution, including the steps of: forming a mixture by combining a sample solution, at least one fluorescent latex microsphere containing a lanthanide chelate and an antibody having specificity for an analyte; placing the mixture onto a sample zone of a solid support matrix, the solid support matrix comprising the sample zone and a capture zone; waiting a pre-determined time for the mixture to move across the solid support to the capture zone; and detecting the presence of an analyte in the sample solution by measuring the amount of fluorescence on the capture zone.

Brief Description of the Drawings

FIGURE 1 is a logarithmic plot illustrating the results of Experiment 1 wherein the fluorescence of four standard solutions of human Chorionic Gonadotropin was determined over six separate observations.

FIGURE 2 is a logarithmic plot illustrating the results of Experiment 2 wherein the fluorescence of fourteen standard solutions of human Chorionic Gonadotropin was determined over 3 separate observations.

FIGURE 3 is a linear regression line plot of the Radio Immunoassay (RIA) vs. fluorescence results of Experiment 2.

Detailed Description of the Preferred Embodiment

The present invention relates to a one-step immunoassay method and system for rapidly and accurately determining the level of a target analyte in a sample solution. One embodiment of the invention is a one-step bioaffinity assay wherein a fluorescent latex microsphere containing an aromatic diketone chelate of certain rare earth

metals is used as the label. Lanthanide chelates, such as europium, terbium and samarium are examples of aromatic diketone chelates.

Because the aromatic diketone chelates of rare earth metals such as europiumbenzoylacetonate and europiumbenzoyltrifluoracetonate have a relatively long-lived fluorescence, they can be used to detect a minute quantity of analyte in a sample. In addition, the Stoke's shift is very long (240-270nm) so that the band width for these labels is very small.

In general, an immunoassay incorporating embodiments of the invention is made by first mixing a suspension containing fluorescent latex microspheres having incorporated europium chelates with antibodies against the target analyte. The antibodies become passively bound to the latex microspheres and are thereafter lyophilized onto the labeling zone of a solid support matrix.

When a sample to be tested is added to the sample zone of the solid support matrix, the sample is carried over the labeling zone by capillary action. Any analyte in the sample becomes immunologically bound to the target antibodies on the surface of the latex microsphere. The microsphere/analyte complex then continues its migration through the solid support matrix.

The sample zone serves to begin the flow of analyte-containing sample, and typically will be constructed of a material that exhibits low analyte retention. One means for imparting this property involves impregnating the sample zone with a neutral protein-blocking reagent, followed by treatment to immobilize the blocking agent (e.g., lyophilization). An additional advantage of this treatment is the increased wetability and wicking action which speeds transfer of the liquid sample into the labeling zone. The sample zone may also function as a mechanical filter, entrapping any undesirable particulates present in the sample solution.

The solid support matrix also contains a capture zone that can preferably include a capture line of antibodies. The antibodies in the capture zone are normally chosen to bind with a second epitope on the target analyte. The target analyte thereby becomes concentrated at the capture line by binding to the thin line of antibodies on the solid support matrix instead of a broad patch of antibodies. As the microsphere/analyte complex is carried over the capture zone, the second epitope on the analyte becomes bound to the antibodies at the capture line. As a result, the capture line becomes fluorescent if the target analyte is present in the sample. By placing the capture antibodies on the solid support matrix in a thin line, the immunoassay system can detect very minute quantities of analyte in the sample. For example, an immunoassay of the present invention can detect as little as 1.5 picograms of analyte in a sample.

Because each molecule of analyte can bind to a fluorescent latex microsphere, we found a monotonically increasing relationship between the concentration of analyte in the sample and the concentration of fluorescent microspheres bound at the capture line. Thus, a sample containing the target analyte will produce a fluorescent band across the capture line of the solid support matrix with a brightness that is directly proportional to the quantity of analyte in the sample.

To reduce the amount of background fluorescent noise from unbound fluorescent microspheres in the solid support matrix, a polycarbonate carrier can be used to hold the solid support matrix as it is being read in the

fluorimeter. The polycarbonate carrier can include a slit on both sides of the solid support matrix corresponding to the position of the capture line. The carrier and solid support matrix can then be exposed to a fluorescent excitation pulse of light conducted through the first slit in the carrier. In one embodiment, a Xenon flash lamp is used to deliver the excitation pulse. One known Xenon flash lamp is produced by Hamamatsu Corporation. A band pass filter can be placed between the light source and carrier to eliminate counting light pulses of the incorrect wavelength.

The emitted light from the capture line can then be passed through a second slit in the carrier to a photon counting detector. One such photon counting detector is made by Hamamatsu Corporation. In addition, a band pass filter can be positioned between the carrier and photon counting detector to help eliminate any stray fluorescence from the naturally fluorescing matrix constituents. Because the capture line provides a narrow band of high intensity fluorescence, sensitivities of analyte in the 1.5 to 2 picogram range have been achieved.

Miniaturization of the solid support matrix and diagnostic device also contributes to the remarkable speed of the assay. Miniaturization permits instantaneous results which are observable as soon as the sample contacts the capture zone and which occur almost immediately, or within 60 seconds, of adding to the sample receiving zone. The speed of appearance and intensity of the positive visible reaction seen depends on the concentration of analyte in the sample. The speed of appearance of the positive visual reaction can be adjusted to provide the optimal visual result with concentrations of analyte of clinical importance and adjusted to suit the timing needs of the end-user.

Suitable analytes detectable by the invented immunoassay devices are any for which a specific binding partner can be found. In general, most analytes of medical and biological significance can find specific binding partners in antibodies prepared against them or fragments of these antibodies. Suitable analytes include soluble analytes such as hormones, enzymes, lipoproteins, bacterial or viral antigens, immunoglobulins, lymphokines, cytokines, drugs, soluble cancer antigens, and the like. Also included as suitable analytes are hormones such as human chorionic gonadotropin (hCG), insulin, glucagon, relaxin, thyrotropin, somatotropin, gonadotropin, follicle-stimulating hormone, gastrin, bradykinin, vasopressin, and various releasing factors. A wide range of antigenic polysaccharides can also be determined such as those from Chlamydia, Neisseria gonorrheae, Pasteurella pestis, Shigella dysentereae, and certain fungi such as Mycosporum and Aspergillus. Another major group comprises oligonucleotide sequences which react specifically with other oligonucleotides or protein targets. An extensive list of soluble analytes determinable in the method of the invention is found in U.S. Patent No. 3,996,345, which is incorporated herein by reference.

The solid support matrix can be disposed within a housing that is both protective and functional, as described below. In one preferred embodiment the housing is adapted to have at least one port for receiving a liquid sample and guiding fluid flow of the sample solution to contact the solid support matrix at the sample receiving zone. The housing also can have windows to allow measurement of the fluorescence at the capture zone, and which allow a user to view portions of the solid support matrix, including portions of the capture zone and/or the absorbent zone. The following experiment illustrates one embodiment of a method for practicing the invention.

EXPERIMENT 1

We performed a first experiment to determine whether minute quantities of human chorionic gonadotropin (hCG) could be detected in urine as an assay for pregnancy. It would be advantageous to provide an assay having the ability to detect minute quantities of hCG in order to determine whether or not a woman was pregnant at the earliest possible date.

For this experiment, a one-step solid support matrix was constructed as described in Pawlak, J., patent application No. WO94/01775 published January 20, 1994, which is incorporated herein by reference. The solid support matrices had a sample zone and a capture zone. Disposed on the capture zone was a capture line of anti-hCG antibodies. Europium microspheres (Emerald Diagnostics) were diluted to 2.5% from a stock 10 weight percentage suspension and thereafter passively coupled with a 2 weight percentage solution of anti-alpha human chorionic gonadotropin (hCG) monoclonal antibody and mixed at room temperature on a rotator for 4 hours. The microspheres were centrifuged at 10,000 rpm and resuspended in an equivalent replacement volume of a 10 weight percentage solution of methylated bovine serum albumen (BSA). The BSA albumen was used to block any non-specific binding of the anti-hCG monoclonal antibody.

The resuspended solution was thereafter placed on a rotator for 4 hours and re-centrifuged at 10,000 rpm. The resulting solution was resuspended in BSA as before. The suspended microspheres were then diluted to 0.5 weight percentage with a BSA solution. Control wet assays were then performed by preparing serial dilutions from stock hCG solutions established to be 24,000 mIU/mL by a radioimmunoassay. The various hCG dilutions were mixed with the 0.5 weight percentage suspension of europium microspheres and anti-hCG antibody so that the microsphere solution became a final concentration of 0.05 weight percentage in the hCG dilution.

One hundred microliters of each diluted suspension was added to the sample zone of a solid support matrix. The immunological reaction was allowed to proceed for 3 minutes and thereafter the solid support matrix was loaded into a polycarbonate carrier. The carrier had a linear opening corresponding with the position of the capture line on the solid support matrix so that the capture line would be exposed to the fluorescent lamp while inside the carrier. The carrier and solid support matrix were then nested inside a cylindrical optical chamber of the fluorescent instrument. Fluorescent counts were registered as discriminated charged pulses at the photon counter and integrated over a 10 millisecond interval.

A ten microsecond excitation pulse was delivered by a Hamamatsu Xenon flash lamp which was signaled from a pulse generator. It should be noted that the delivery rate of the pulse could be adjusted from a single pulse to 4 pulses over the 10 millisecond integration period to optimize the signal-to-noise ratio. An ND-2 band pass filter, which allowed approximately 1% of the admitted fluorescence to the detector, was added to the emission side of the optical path in order to avoid photosaturation that might impair the response linearity.

Data from the photon counter was recorded over a one minute period by a computer linked to the photon counter and thereafter written to an ASCII file on a spreadsheet. For one dataset, the average count value at each concentration of hCG was determined on 6 replicant measurements and the standard deviation, mean and percent CV was determined at each level. The data from this experiment is shown in Table 1 below.

TABLE 1

Fluorescent Counts of hCG Standard Samples

	A(X) mIU/mL	B Obs. 1	C Obs. 2	D Obs. 3	E Obs. 4	F Obs. 5	G Obs. 6	mean(Y)	sd(yEr±)	se(yEr±)
1	240	1395.2	1258.4	2289	1264	2220	1740.8	1694.56667	468.3627	191.20827
2	24	909.6	823.4	1020	595.2	991.2	990	888.23333	160.53004	65.53612
3	2.4	263.2	352	200	368.8	294.4	288.8	294.53333	61.26855	25.01278
4	0.24	172	192.8	220.8	208.8	201.6	226.4	203.73333	19.80815	8.08664
5	0	168	169.6	158	156	152.8	140.8	157.53333	10.58427	4.32101

Figure 1 is a logarithmic plot illustrating the correlation between the average number of fluorescent counts from the capture line of a solid support matrix and the concentration of hCG analyte in a sample. The average number of fluorescent counts was based on the mean of the 6 observations listed in Table 1 at each analyte concentration.

As shown in Figure 1, a linear relationship was found between the quantity of hCG in the standards and the amount of fluorescence at the capture line. Thus, the $\log_{10}$(hCG) related to the $\log_{10}$(fluorescent counts).

Experiment 2

In another experiment, the relationship between the amount of fluorescence and the quantity of an analyte was measured over a wider range of hCG values. In this experiment, standard samples containing from 0.15mIU/mL hCG to 2,400mIU/mL hCG were run on solid support matrices as discussed in Experiment 1. An average value of fluorescence for each standard solution was determined by performing three separate trials with each concentration of the hCG analyte. The results of this experiment are listed in TABLE 2 below.

TABLE 2

Fluorescence of Standard Sample Solutions

	A(X) mIU/mL hCG	B 2/4/97	C 1/27/97	D 3/4/97	E Pred. 2/4/97	F Pred. 1/27/97	G Pred. 3/4/97	H(Y) Av. Pred.
1	0	101.2	75.24	157.5	0.02	0.02	0.062	0.021
2	0.12	--	120.2	--	--	0.09	--	0.09
3	0.15	182.3	--	--	0.16	--	--	0.16
4	0.24	--	--	203.7	--	--	0.36	0.36
5	1	--	242	--	--	0.5	--	0.5
6	1.5	365	--	--	1.46	--	--	1.46
7	2.4	--	436	294	--	2.82	1.11	1.97
8	3	--	574	--	--	6.33	--	6.33
9	4.8	--	829.9	--	--	18.64	--	18.64
10	6	540.8	--	--	5.07	--	--	5.07
11	24	793.3	--	888	17.07	--	33.68	25.38
12	48	1344.4	--	--	90.92	--	--	90.92
13	240	1730	1259.2	1694.6	202.25	63.28	247.48	171
14	2400	--	4943.2	--	--	3485.39	--	3485.39

Figure 2 is a logarithmic plot illustrating the linear relationship we discovered between the quantity of hCG in the standard samples and the amount of fluorescence detected by the photon counter.

The data from TABLE 2 were composited and the resulting slope and intercept determined on a regression line through the fluorescence-predicted values versus the nominal radio immunoassay assigned values. The resulting graph and line statistics are illustrated in Figure 3. Based on the foregoing dose-response curve shown in Figure 3, we calculated that the background fluorescence of the solid support matrix was equivalent to approximately 0.025

mIU/mL. Based on the linear nature of the log-log plots, a two-point calibration procedure by running known concentrations of analyte concurrently with a sample under test would provide for a quick, accurate assay.

EXPERIMENT 3

A test for hCG is run to determine whether a woman is pregnant. Approximately one-hundred microliters of urine is placed onto the sample zone of a solid support matrix, as discussed above in Experiment 1. The solid support matrix also includes two hCG standard lanes with 0.15mIU/mL hCG and 8 mIU/mL hCG, respectively. By having standard solution lanes on each one-step immunoassay, a logarithmic chart of the immunofluorescence counts vs. analyte concentration can be prepared for each assay. The amount of analyte in the sample solution can then be determined from the logarithmic chart.

The immunological reaction is allowed to proceed for 3 minutes and thereafter the solid support matrix is loaded into a polycarbonate carrier. The carrier and solid support matrix are then nested inside a cylindrical optical chamber of the fluorescent instrument. Fluorescent counts are registered as discriminated charged pulses at the photon counter and integrated over a 10 millisecond interval.

A logarithmic plot of the number of fluorescent counts from the standard solutions in relation to the quantity of hCG is prepared. The amount of hCG in the urine sample is calculated by plotting the number of fluorescent counts from the sample to this plot. The woman is determined to be pregnant because her urine sample contains over 100 mIU/mL hGC.

It should be noted that the present invention is not limited to only those embodiments described in the Detailed Description. Any embodiment which retains the spirit of the present invention should be considered to be within its scope. However, the invention is only limited by the scope of the following claims.

WHAT IS CLAIMED IS:

1. A one-step immunoassay device, comprising:

a solid support matrix having a sample zone, a label zone and a capture zone; and

at least one fluorescent latex microsphere containing a lanthanide chelate disposed on said label zone

2. The immunoassay device of Claim 1, wherein said fluorescent latex microsphere additionally comprises an antibody.

3. The immunoassay device of Claim 2, wherein said antibody is a monoclonal antibody.

4. The immunoassay device of Claim 3, wherein said monoclonal antibody binds with high affinity to human Chorionic Gonadotropin (hCG).

5. The immunoassay device of Claim 1, wherein said lanthanide chelate is selected from the group comprising: terbium, europium and samarium.

6. The immunoassay device of Claim 1, wherein said capture zone additionally comprises a capture line of antibodies.

7. The immunoassay device of Claim 1, wherein said solid support matrix comprises nitrocellulose.

8. A lateral flow immunoassay device having a solid support matrix, comprising:

a sample zone;

a label zone in fluid communication with said sample zone, said label zone further comprising at least one fluorescent latex microsphere containing a lanthanide chelate in communication with an antibody having specificity for an analyte; and

a capture zone in fluid communication with said label zone, said capture zone containing a capture antibody having affinity for said analyte.

9. The immunoassay device of Claim 8, wherein said antibody is a monoclonal antibody.

10. The immunoassay device of Claim 9, wherein said monoclonal antibody binds with high affinity to human Chorionic Gonadotropin (hCG).

11. The immunoassay device of Claim 8, wherein said lanthanide chelate is selected from the group comprising: terbium, europium and samarium.

12. The immunoassay device of Claim 8, wherein said capture zone additionally comprises a capture line of antibodies.

13. The immunoassay device of Claim 8, wherein said solid support matrix comprises nitrocellulose.

14. A method for detecting the presence of an analyte in a sample, comprising the steps of:

a) contacting a sample zone on a solid support matrix with a sample to be tested for the presence of an analyte, wherein said sample moves along said solid support such that the sample contacts a label zone comprising antibodies with specificity for said analyte in association with fluorescent latex microspheres having a lanthanide chelate, and wherein said analyte, said antibodies and said fluorescent latex microspheres form a fluorescent complex; and

b) detecting the presence of said fluorescent complex, wherein the detection of said fluorescent complex is indicative of said analyte being present in said sample.

15. The method of Claim 14, wherein said lanthanide chelate is selected from the group comprising: terbium, europium and samarium.

16. The method of Claim 14, wherein said solid support matrix comprises nitrocellulose.

17. The method of Claim 14, wherein said analyte is human Chorionic Gonadotropin (hCG).

18. The method of Claim 14, wherein step a) further comprises said fluorescent complex moving along said solid support until said fluorescent complex is immobilized with respect to said solid support matrix by contacting a capture zone on said solid support matrix containing antibodies against said analyte.

19. A method for detecting the presence of an analyte in a sample solution, comprising the steps of:

placing a sample solution onto a sample zone of a solid support matrix, said solid support matrix comprising the sample zone, a label zone and a capture zone, wherein and at least one fluorescent latex microsphere containing a lanthanide chelate is disposed on said label zone;

waiting a pre-determined time for said sample to move across said solid support to said capture zone; and

detecting the presence of an analyte in said sample solution by measuring the amount of fluorescence on said capture zone.

20. The method of Claim 19, wherein said lanthanide chelate is selected from the group comprising: terbium, europium and samarium.

21. The method of Claim 19, wherein said solid support matrix comprises nitrocellulose.

22. The method of Claim 19, wherein said analyte is human Chorionic Gonadotropin (hCG).

23. A method for detecting the presence of an analyte in a sample solution, comprising the steps of:

forming a mixture by combining a sample solution, at least one fluorescent latex microsphere containing a lanthanide chelate and an antibody having specificity for an analyte;

placing said mixture onto a sample zone of a solid support matrix, said solid support matrix comprising the sample zone and a capture zone;

waiting a pre-determined time for said mixture to move across said solid support to said capture zone; and

detecting the presence of an analyte in said sample solution by measuring the amount of fluorescence on said capture zone.

24. The method of Claim 23, wherein said lanthanide chelate is selected from the group comprising: terbium, europium and samarium.

25. The method of Claim 23, wherein said solid support matrix comprises nitrocellulose.

26. The method of Claim 23, wherein said analyte is human Chorionic Gonadotropin (hCG).

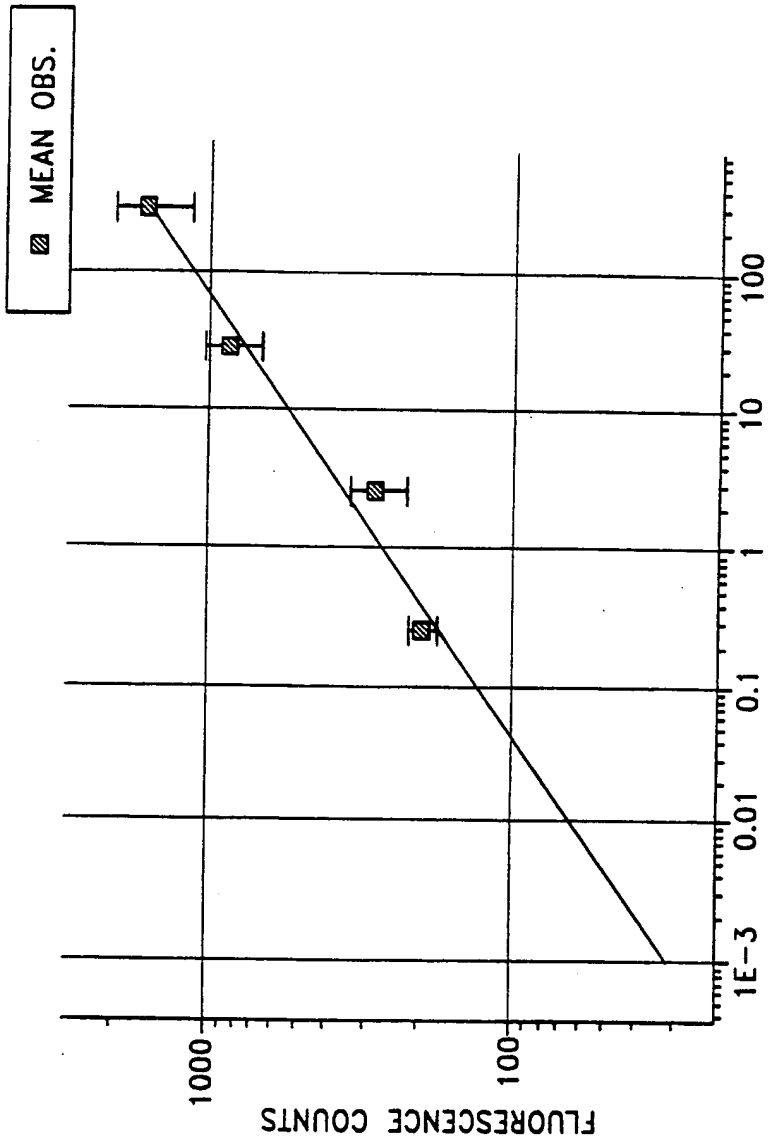

FIG. 1

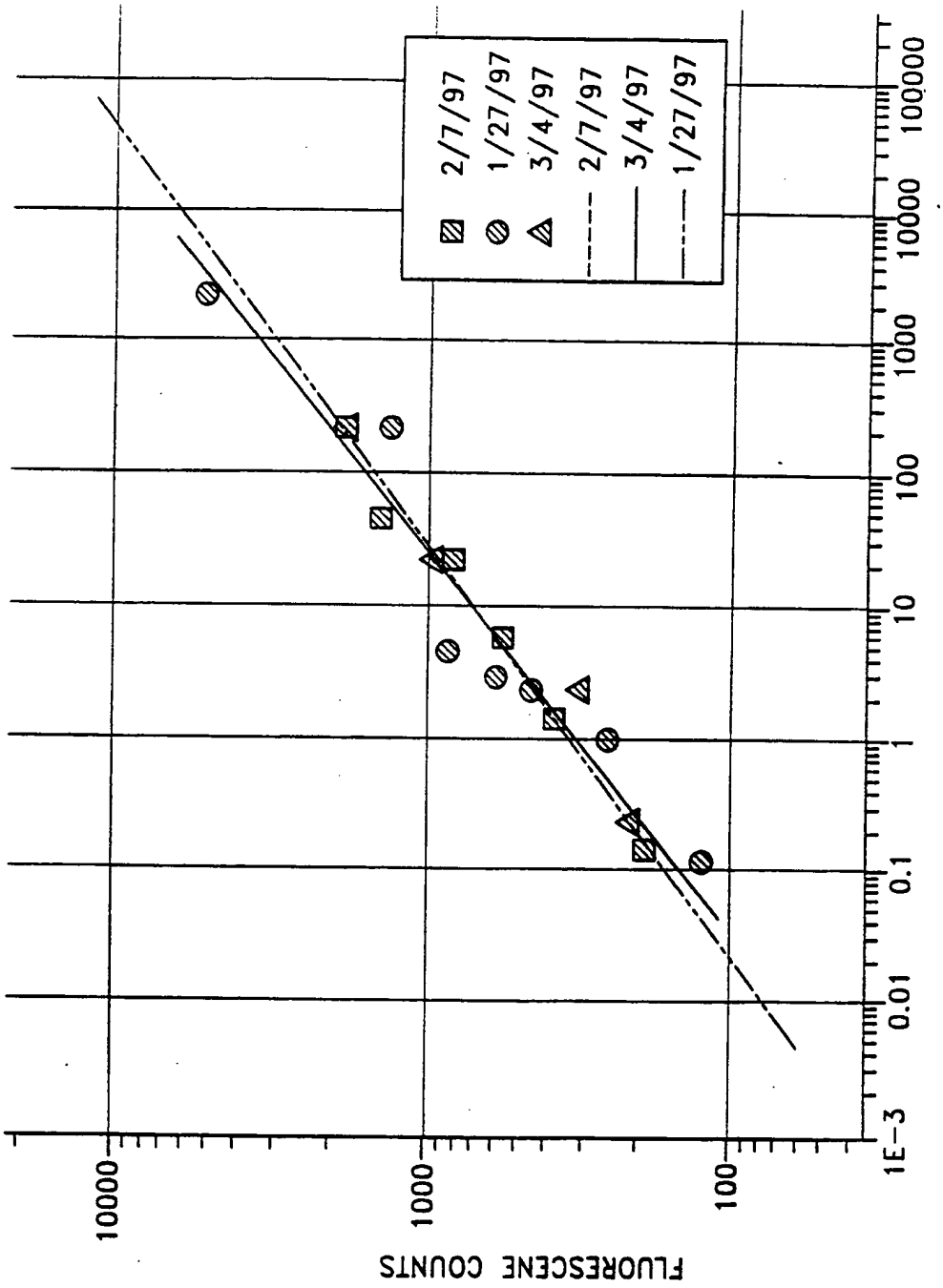
2/7/97
1/27/97
3/4/97
2/7/97
3/4/97
1/27/97
FLUORESCENE COUNTS
10000
1000
100
1E-3
0.01
0.1
1
10
100
1000
10000
100000

FIG. 2

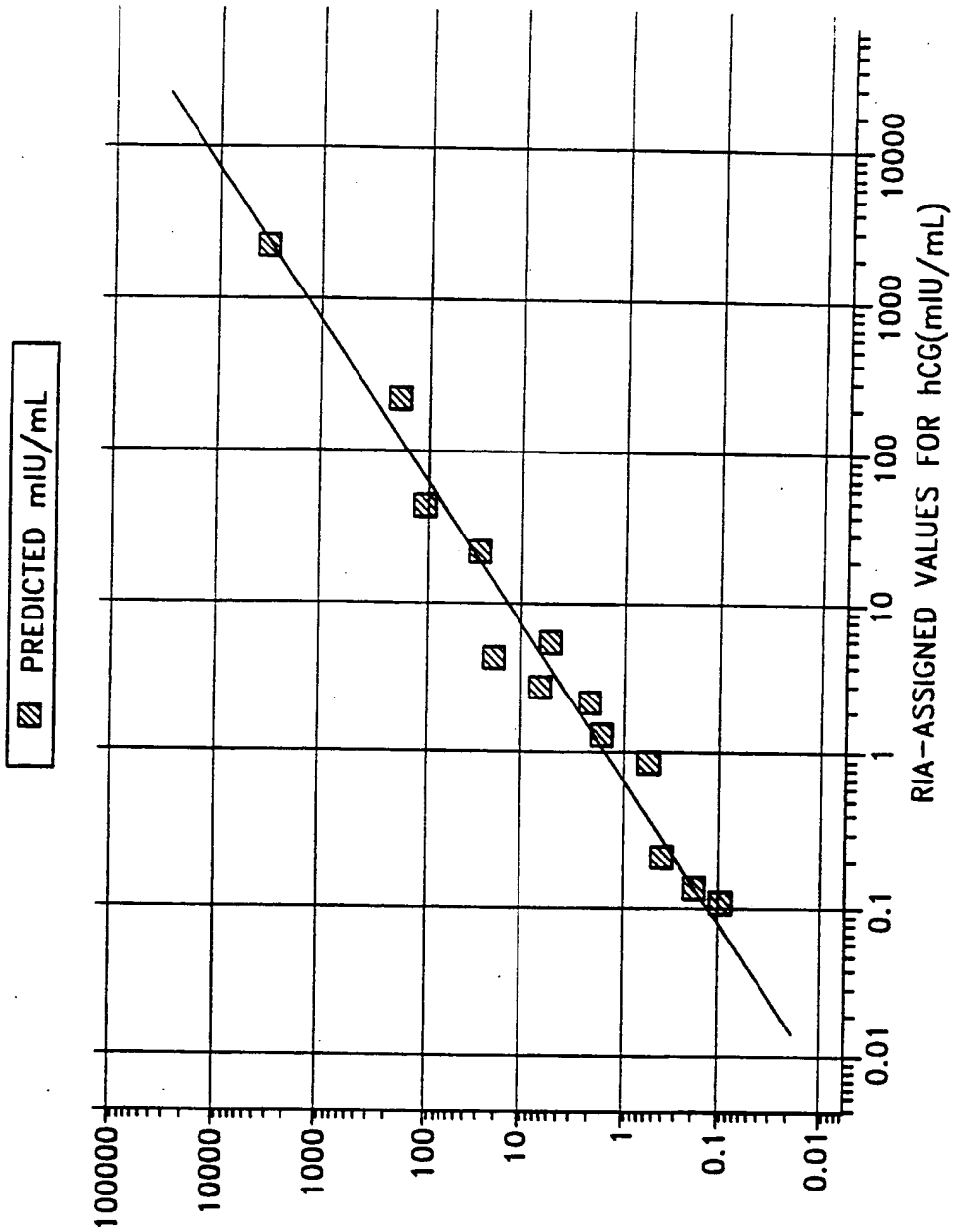
PREDICTED mIU/mL
RIA-ASSIGNED VALUES FOR hCG(mIU/mL)
0.01
0.1
1
10
100
1000
10000
100000
mIU/mL hCG PRED. BY FLUOR.
(BASED ON AVERAGED OBSERVATIONS)

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/07079

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/55, 56, 57, 58, 59, 60, 61; 435/7.1, 7.2, 7.34, 7.92, 7.93, 7.94, 7.96, 287.8, 287.9, 885, 969, 970; 436/518, 528, 530, 541

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, WPIDS, MEDLINE, PALM

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,258,001 A (PIERCE et al) 24 March 1981, see entire document.	1-26
Y	US 4,812,414 A (WARREN, III et al) 14 March, 1989. See entire document.	1-26
Y	US 5,141,850 A (COLE et al) 25 August 1992, see entire document.	1-26
Y	WO 88/08534 A1 (UNILEVER PLC) 03 November 1988, see entire document.	1-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 MAY 1998	Date of mailing of the international search report 18 JUN 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BAO-THUY L. NGUYEN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/07079

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

G01N 21/00, 31/22, 33/544, 33/538, 33/53, 33/567, 33/537, 33/543; C12M 1/00; C12N 1/00, 1/20

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

422/55, 56, 57, 58, 59, 60, 61; 435/7.1, 7.2, 7.34, 7.92, 7.93, 7.94, 7.96, 287.8, 287.9, 885, 969, 970; 436/518, 528, 530, 541